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BIOLOGICAL BULLETIN

EXPERIMENTS WITH CHRYSOMELID BEETLES.

III. The Effects of Killing Parts of the Eggs of *Leptinotarsa decemlineata*.[1]

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Contents.

1. Introduction.

The method used in the experiments described in this paper, *i. e.*, killing parts of the egg, has been employed by a number of investigators in many ways and for many purposes. Heat and electricity are the agents which have been most frequently applied. So far as I know insects' eggs have heretofore never been operated upon in this way. The results obtained by the use of these agents are quite similar to those so frequently brought about by removing parts of the egg or embryo, or by isolating blastomeres. The latter process is of course impossible in the case of the insect's egg, since cleavage is superficial, but material has been removed successfully from different parts of beetles' eggs in various stages of development.[2]

Three years ago a preliminary report was made of experiments in removing portions of the eggs of *Calligrapha multipunctata*,

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 131. Parts I. and II. of "Experiments with Chrysomelid Beetles" appeared in the Biological Bulletin, Vol. XIX., June, 1910, pp. 18–30.

C. bigsbyana, *C. lunata* and *Leptinotarsa decemlineata*.[1] Before the results of those experiments and the data contained in the present contribution can be understood clearly, a brief account of the structure of freshly laid chrysomelid eggs and of the principal stages in their normal embryonic development is necessary.

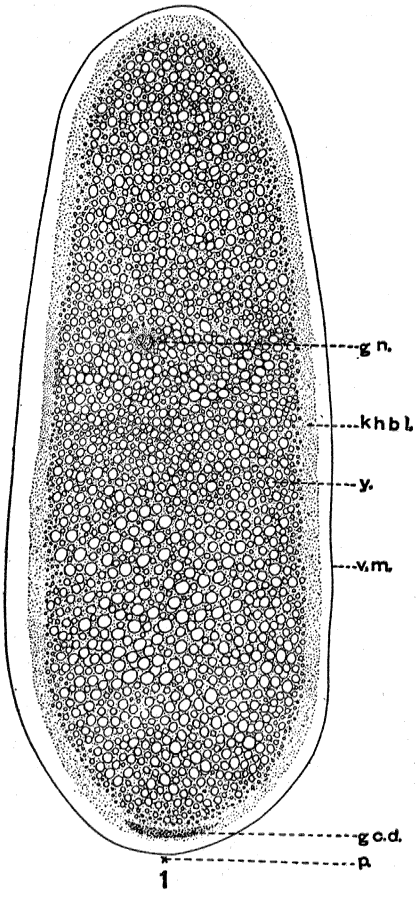

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FIG. 1. A longitu inal section through an egg of *Calligrapha bigsbyana* four hours after deposition. *gcd*, germ cell determinants; *gn*, germ nuclei copulating; *khbl*, "Keimhautblastem"; *p*, posterior; *vm*, vitelline membrane; *y*, yolk. The eggs of *Leptinotarsa decemlineata* are not visibly different from those of *C. bigsbyana*.

The freshly laid egg (Fig. 1) consists of a large central mass of yolk globules (*y*) and a comparatively thin superficial layer of cytoplasm, the "Keimhautblastem" (*khbl*). Two envelopes cover the egg, the vitelline membrane (*vm*) and the chorion. Polar bodies are usually present at this time, and the egg nucleus is in the act of union with the sperm nucleus (*gn*), or else cleavage has already begun. Embedded in the "Keimhautblastem" at the posterior end of the egg is a disc-shaped mass of darkly staining granules, which I have called the pole-disc, or germ cell determinants (*gcd*).

As cleavage progresses, a separation of the cleavage products into sections occurs; the

[1] Hegner, R. W., '08*b*, "The Effects of Removing the Germ Cell Determinants from the Eggs of Some Chrysomelid Beetles," BIOL. BULL., Vol. 16

nuclei of one group form a more or less regular layer equidistant from the periphery, and later migrate outward, fuse with the "Keimhautblastem" and become the blastoderm (Fig. 2, *bl*). The nuclei of the other group remain behind in the yolk (Fig. 2, *v*), which it is their duty to dissolve. Eight of the cleavage products which reach the posterior end do not help to form the blastoderm, but gather the germ cell determinants about them and continue their migration until they are entirely separated from the egg; these are the primordial germ cells (Fig. 2, *pgc*).

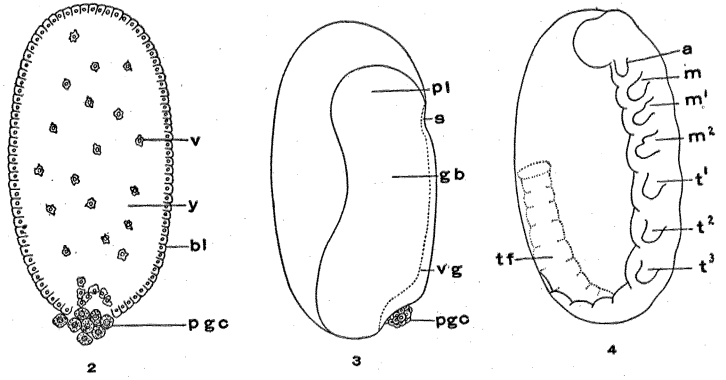

FIG. 2. A longitudinal section through an egg of *Leptinotarsa decemlineata* one day after deposition when in the blastoderm stage. *bl*, blastoderm; *pgc*, primordial germ cells; *v*, vitellophag; *y*, yolk.

FIG. 3. Superficial view of the right side of an egg of *Leptinotarsa decemlineata* thirty-six hours after deposition. *gb*, germ band; *pgc*, primordial germ cells; *pl*, procephalic lobes; *s*, stomodeum; *vg*, ventral groove.

FIG. 4. Surface view of right side of an egg of *Leptinotarsa decemlineata* forty-eight hours after deposition. *a*, antenna; *m*, mandible; *m'*, first maxilla; m^2, second maxilla; t'–t^3, thoracic appendages; *tf*, tail fold.

They remain quiescent for a considerable period and then migrate into the embryo, which has developed in the meantime.

At the end of two days the germ band appears on the ventral surface of the egg (Fig. 3, *gb*); this lengthens within the next twenty-four hours, growing forward to the extreme anterior end, and posteriorly until the tail-fold reaches over half way up on the dorsal surface (Fig. 4, *tf*). During this elongation the embryo segments and the appendages of the head and thorax grow out. The entire embryo then contracts antero-posteriorly and begins to grow around the yolk (Fig. 5). This contraction continues

until the end of the tail-fold coincides with the posterior end of the egg (Fig. 6). The larva (Fig. 7) usually appears in five or six days.[1]

2. Killing the Germ Cell Determinants.

The method used in the experiments mentioned above to remove the germ cell determinants was to prick the posterior end of the freshly laid egg with a needle and allow them to flow out. These experiments were not considered entirely successful, since I was unable to determine in any case whether all of the germ cell determinants had been removed. Some of the embryos which developed from eggs operated upon in this way produced

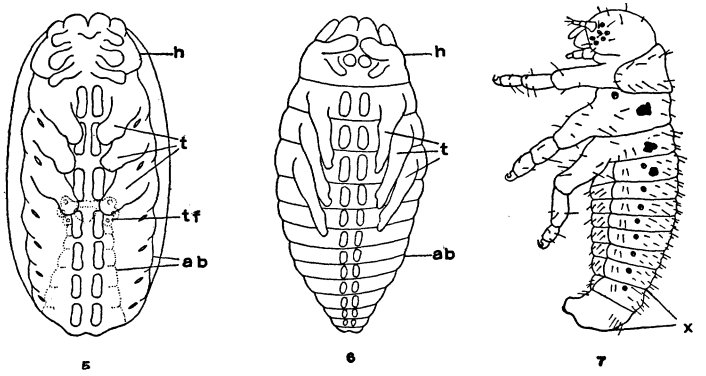

Fig. 5. Ventral view of an egg of *Leptinotarsa decemlineata* sixty hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

Fig. 6. Ventral view of an egg of *Leptinotarsa decemlineata* seventy-two hours after deposition. *ab*, abdomen; *h*, head; *t*, thoracic appendages.

Fig. 7. Side view of a newly hatched larva of *Leptinotarsa decemlineata*.

either a lesser number of germ cells than normal, or else no germ cells at all. The conclusion was reached that if all of the germ cell determinants are removed from the egg no germ cells will be produced, and that the granules which constitute the pole disc are really germ cell determinants.[2]

Two series of experiments (L.D. 09 and L.D. 018) were carried

[1] Hegner, R. W., '08*a*, "Observations on the Breeding Habits of Three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*, *Psyche* Vol. 15.

[2] For a discussion of this subject see Hegner, R. W., '11, "The Germ Cell Determinants in the Eggs of Chrysomelid Beetles," *Science*, Vol. XXXIII., pp. 71–72.

out in order to learn whether or not germ cells would appear in embryos if the germ cell determinants were prevented from taking part in their development. Certain data regarding these experiments are listed in Tables I. and II. Eggs were oriented as soon as laid and the central region of the posterior end of each was touched with a hot needle, thus killing the protoplasm just beneath in which the germ cell determinants were embedded. Since it was impossible to see the germ cell determinants in the living egg, a rather large area had to be killed in order to be certain that all of them had been reached. As noted above, the experiments in removing the germ cell determinants by pricking the egg and allowing them to flow out were not entirely successful because it was never possible to tell whether all of them had been obtained.

TABLE I.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 09.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 09 A	Control.			Hatched in 6 days.
L.D. 09 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	4 cleavage nuclei present.
L.D. 09 B2			1 day.	See Fig. 8.
L.D. 09 B3			2 days.	
L.D. 09 B4			3 days.	See Fig. 11.
L.D. 09 B5			4 days.	
L.D. 09 B6			5 days.	
L.D. 09 B7			7 days.	

TABLE II.

EXPERIMENTS IN KILLING THE GERM CELL DETERMINANTS.

Leptinotarsa decemlineata—Series L.D. 018.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 018 A	Control			Hatched normally.
L.D. 018 B1	Immediately after deposition (see Fig. 1).	Posterior end killed with hot needle.	0	2 cleavage nuclei found.
L.D. 018 B2			1 day.	
L.D. 018 B3			2½ days.	See Fig. 9.
L.D. 018 B4			3 days.	
L.D. 018 B5			4 days.	
L.D. 018 B6			6 days.	

Many of the eggs operated upon in experiments L.D. 09 and L.D. 018 did not develop at all or developed abnormally. Under

ordinary circumstances eggs sometimes become shapeless masses of tissue or remain in the condition of the freshly laid egg, but this fact does not explain the great number of cases observed among the operated eggs. It is evident that many of the operated eggs failed to develop because of the conditions of the experiments. For this reason only a few of the best defined embryos have been selected for descriptive purposes.

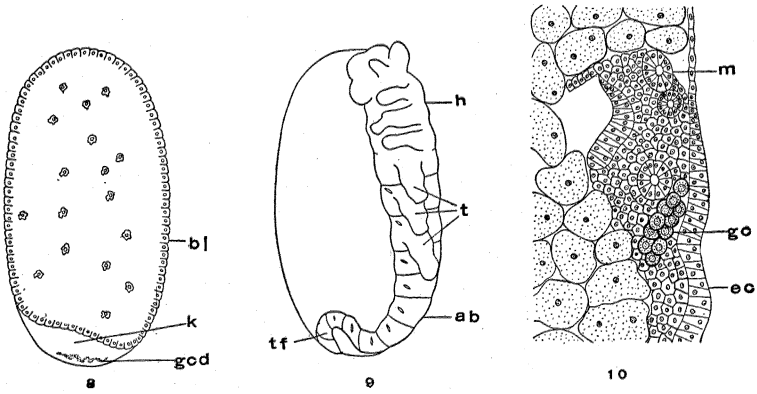

FIG. 8. Longitudinal secion through an egg of *Leptinotarsa decemlineata* (L.D. 09 B2). The posterior end was killed with a hot needle just after the egg was laid (see Fig. 1); the egg was then allowed to develop for twenty-four hours. *bl*, blastoderm; *gcd*, germ cell determinants; *k*, portion of egg killed.

FIG. 9. Side view of an egg of *Leptinotarsa decemlineata* (L.D. 018 B3). The posterior end was killed with a hot needle just after deposition (see Fig. 1); the egg was then allowed to develop for sixty hours. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

FIG. 10. Longitudinal section through the tail fold of a normal embryo of *Leptinotarsa decemlineata* sixty hours old, showing the germ cells (*gc*). *ec*, ectoderm; *m*, malpighian tubules.

Fig. 8 is from a longitudinal section of an egg (L.D. 09 B2) which was operated upon just after deposition and was then allowed to develop for twenty-four hours. This should be compared with the normal egg at a similar stage of development (Fig. 2). In the egg shown in Fig. 8 the germ cell determinants (*gcd*) and a portion of the neighboring yolk and cytoplasm (*k*) have been prevented from taking part in development. That part of the egg which remained alive produced a blastoderm of a single layer of cells (*bl*). The principal difference to be noted

between this preparation and that of a normal embryo is the absence of the primordial germ cells at the posterior end (see Fig. 2, *pgc*). Three perfect series of sections were cut from eggs at this stage, but none disclosed any germ cells.

Fig. 9 is a sketch of embryo L.D. 018 B3 (see Table II.). It was killed two and one half days after the operation and is a stage a trifle younger than that shown in Fig. 5. The tail fold (*tf*), however, is not fully developed as in Fig. 5. Longitudinal sections were cut through two embryos like that shown in Fig. 9, but no germ cells could be found. Fig. 10 was drawn from a section through the tail fold of a normal embryo two and one half days old; it indicates where the germ cells (*gc*) are situated at this time. If germ cells had been present in the embryo shown in Fig. 9, they would certainly have been found.

These experiments demonstrate that germ cells are not produced when the extreme posterior end of the egg is prevented from taking part in development, and it seems probable from the method of origin of the germ cells that the destruction of the germ cell determinants is the real cause of their absence.[1]

3. Killing the Primordial Germ Cells.

Table III. gives the data for the experiments performed in series L.D. 011. Sixty-nine eggs were laid at 11 A.M. June 24,

TABLE III.

Experiments in Killing the Primordial Germ Cells.

Leptinotarsa decemlineata—Series L.D. 011.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 011 A	Control.			Hatched normally.
L.D. 011 B1	One day after deposition (see Fig. 2).	Posterior end killed with hot needle.	0	Like Fig. 2.
L.D. 011 B2			1 day.	
L.D. 011 B3			2 days.	
L.D. 011 B4			3 days.	
L.D. 011 B5			4 days.	
L.D. 011 B6			5 days.	One hatched.
L.D. 011 B7			6 days.	Several hatching.

[1] The writer's papers on "The Origin and Early History of the Germ Cells in some Chrysomelid Beetles" (*Journ. Morph.*, Vol. 20) and on "Germ Cell Determinants and Their Significance" now in press (*American Naturalist*) discuss these points fully.

and were allowed to develop until 11 A.M. June 25. The posterior end of sixty-five of them was then killed with a hot needle. The four controls hatched on June 29. The eggs when operated upon were in a stage like that shown in Fig. 2. One of the operated eggs hatched on June 30 (L.D. 011 B6), several were hatching on the following day, and a number of others were ready to hatch at that time. The one that hatched and three of those ready to hatch were examined and then sectioned and stained. Superficially they resembled the larva shown in Fig. 7, the only difference being the absence of the last two posterior segments which are indicated by the letter *x* in Fig. 7; one possessed all but the last segment. The sections showed that none of these larvæ contained germ cells.

It is evident from these experiments that the primordial germ cells were killed by the operation and no new ones were produced by the developing embryos. This is, I believe, the earliest stage at which surgical castration has been performed among the Insecta. The influence of this operation upon secondary sexual characters could not be determined, since none of these characters make their appearance in the larvæ.

4. Killing Parts of Freshly Laid Eggs.

The experiments described under heading 2, "Killing the Germ Cell Determinants," might be included in this part of the paper, but they were considered of sufficient importance to warrant a special account. In performing the experiments in series L.D. 09 (Table I.) it was found impossible to regulate with any degree of exactness the amount of the egg killed, and, since it was absolutely necessary that all of the germ cell determinants be killed, a larger portion of the egg was killed than desired. Some of these eggs, however, when allowed to continue their development, provided data with regard to the effects of killing a large part of the posterior end of freshly laid eggs.

Fig. 11 was drawn from an egg from series L.D. 09 B4. The portion killed by the operation is labelled *k*; the material that remained alive produced the head (*h*) and part of the thorax (*t*) of an embryo. These parts resemble the corresponding parts of a normal embryo at a like age (see Fig. 6). Apparently that

part of the "Keimhautblastem" which remained alive after the operation, became supplied with nuclei, was broken up into cells, and proceeded to develop that particular part of the embryo to which it would have given rise if the rest of the egg had not been killed.

Two other series of experiments, L.D. 07 and L.D. 08, were performed to test these results and in every case the living part of the egg developed as though the entire egg were intact. None of the tissue that is normally produced by the killed portion was regenerated by the living material. The embryos developed up to the time of hatching, but were unable to break out of the chorion.

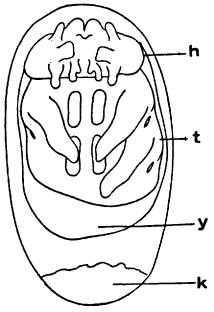

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FIG. 11. Ventral view of an egg of *Leptinotarsa decemlineata* (L.D. 09 B4). The posterior end (*k*) was killed just after deposition (see Fig. 1); a normal head (*h*) and part of the thorax (*t*) developed from the material which remained alive. *y*, yolk.

5. KILLING PARTS OF EGGS IN THE BLASTODERM STAGE.

The eggs used for the experiments designated as series L.D. 04 (Table IV.) were laid at 4 P.M. on June 16, and operated upon at 4 P.M. June 17. They were, at the time of the operation, in a stage similar to that of the egg shown in Fig. 2. As indicated in Table IV., two kinds of operations were performed; the anterior part of one half of the eggs was killed with a hot needle, and the posterior part of the other half was killed in a like manner. Three preparations have been selected to show the results of these experiments, (1) L.D. 04 A2, Fig. 12, (2) L.D. 04 A3, Fig. 13, and (3) L.D. 04 B3, Fig. 14.

The embryos shown in Figs. 12 and 13 developed from eggs which had their posterior parts killed, and were fixed four days and seven days later respectively. One of these embryos (Fig. 12) consists of a head and thorax which appear to be normal in every respect, and are as fully developed as these parts in a normal embryo at a similar age (five days). The abdomen of this

TABLE IV.

EXPERIMENTS IN KILLING PARTS OF EGGS IN THE BLASTODERM STAGE.
Leptinotarsa decemlineata—Series L.D. 04.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 04 A1	Control.			Hatched normally.
L.D. 04 A2	Blastoderm stage (see Fig. 2).	Posterior part killed.	4 days.	See Fig. 12.
L.D. 04 A3			7 days.	See Fig. 13.
L.D. 04 B1		Anterior part killed.	0	
L.D. 04 B2			3 days.	
L.D. 04 B3			4 days.	See Fig. 14.
L.D. 04 B4			7 days.	

embryo is entirely missing. The conclusion is reached that the part of the blastoderm which would have produced the abdomen of the embryo was killed in the operation, and that none of this region was regenerated by the tissue which remained alive.

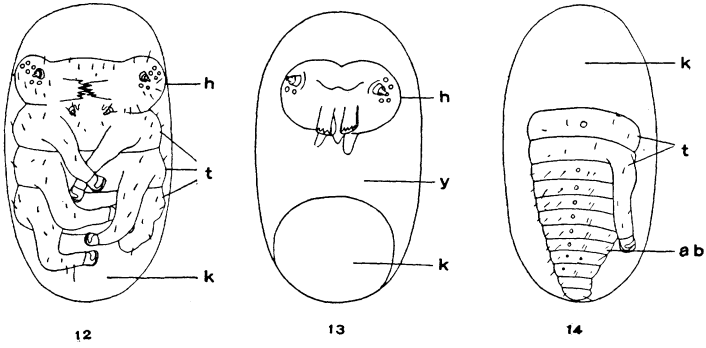

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FIG. 12. Ventral view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 A2). The posterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal head (*h*) and thorax (*t*).

FIG. 13. Ventral view of an egg of *Leptinotarsa decemlineata* eight days old (L.D. 04 A3), operated upon as in Fig. 12. Only a head (*h*) developed from the tissue which remained alive.

FIG. 14. Side view of an egg of *Leptinotarsa decemlineata* five days old (L.D. 04 B3). The anterior end of the egg (*k*) was killed when in the blastoderm stage (see Fig. 2); the blastoderm which remained alive produced a normal abdomen (*ab*) and part of the thorax (*t*).

Fig. 13 was drawn from an egg that was fixed three days later than that of Fig. 12, *i. e.*, at the age of eight days. Only the head of this embryo developed. It is apparent that not only

the tissue destined to produce the abdomen, but also that set aside to form the thorax was killed by the operation. The region of the blastoderm which normally develops into the head covers a considerable area at the time the operation was performed. This area lessens in extent when the germ band arises (Fig. 3, *pl*), and, after the cephalic appendages appear (Fig, 4, *a*, *m*, m^1, m^2), the anterior end of the embryo shortens until the mouth parts are closely crowded together (Figs. 5, 6 and 7). In egg L.D. 04 A3 (Fig. 13) the shortening of the embryo has resulted in the uncovering of a large yolk area (*y*), and, though a comparatively small part of the egg was killed (*k*), this portion bore the blastoderm which in normal eggs gives rise to the larger part of the embryo, *i. e.*, the thorax and abdomen.

When the blastoderm surrounding the anterior end of the egg is killed, only the posterior embryonic region develops from the part which remains alive. This is shown in egg L.D. 04 B3, Fig. 14. Here the normal number of abdominal segments appear as well as two thoracic segments (*t*), one of which has developed a normal pair of legs.

6. Killing Parts of Young Embryos.

The series of figures numbered 15 to 18 show what takes place when parts of young embryos are killed and are thus prevented from continuing development. Table V. gives the data of the operations. The eggs, fifty-two in number, were laid at 10 A.M. June 26; the operations were performed at 10 A.M. June 28, at which time the eggs bore embryos similar to that shown in Fig. 4. Four of the eggs were kept as controls; these hatched on July 2. Approximately one half of the anterior end of twenty-four of the eggs was killed with a hot needle; the posterior half of the other twenty-four eggs was killed in like manner. In every instance the part of the embryo which remained alive developed as though the egg had not been disturbed.

Figs. 15 and 16 show two stages in the development of the posterior part of the embryo, and Figs. 17 and 18 show corresponding stages in the development of the anterior part of the embryo. It is interesting to note that in the egg shown in Fig. 15 not only the anterior end of the embryo, but also the extreme

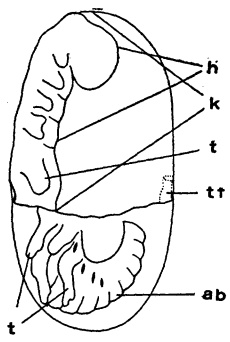
TABLE V.

EXPERIMENTS IN KILLING PARTS OF YOUNG EMBRYOS.

Leptinotarsa decemlineata—Series L.D. 016.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 016 A	Control.			Hatched normally.
L.D. 016 B1	Young embryo (see Fig. 4).	Anterior part killed.	0	See Fig. 4.
L.D. 016 B2			1 day.	See Fig. 15.
L.D. 016 B3			2 days.	
L.D. 016 B4			3 days.	
L.D. 016 B5			4 days.	See Fig. 16.
L.D. 016 C1		Posterior part killed.	1 day.	See Fig. 17.
L.D. 016 C2			2 days.	See Fig. 18.
L.D. 016 C3			3 days.	
L.D. 016 C4			4 days.	

end of the tail fold (*tf*) was killed by the operation, and that the intermediate region consisting of two thoracic segments and eight abdominal segments continued to develop normally except for the mechanical difficulties interposed by the killed material.



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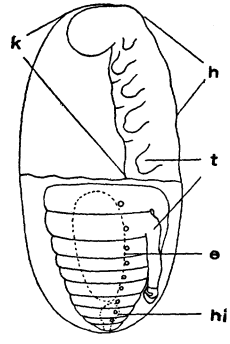

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FIG. 15. Side view of an egg of *Leptinotarsa decemlineata* three days old. (L.D. 016 B2). The anterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the posterior end continued to develop. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold.

FIG. 16. As in Fig. 15, six days old (L.D. 016 B5). *e*, enteron; *hi*, hind intestine.

An *in toto* preparation of an older embryo from this material (Fig. 16) shows that the living part of the embryo succeeded in growing around the yolk and developing a hind intestine (*hi*) which grew forward toward the yolk-filled enteron (*e*).

The preparation shown in Fig. 17 is from an egg fixed one day after the posterior end had been killed, and is the same age as that of Fig. 15, *i. e.*, three days old. It is of special interest, since the end of the tail fold (*tf*), which was not killed by the operation, has continued to develop, although it is an extremely small piece of tissue and was separated from the rest of the living embryo by a considerable amount of yolk. The anterior part of the embryo consisting of the cephalic region and the first thoracic segment, developed normally. During the twenty-four hours between the operation and fixation, the living part of the

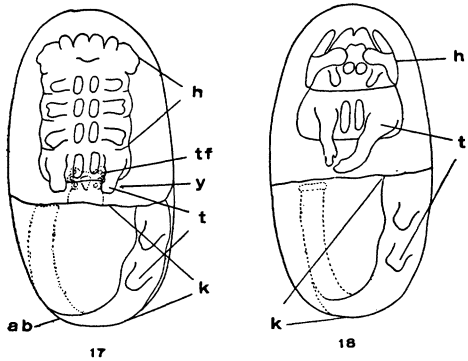

FIG. 17. Side view of an egg of *Leptinotarsa decemlineata* three days old (L.D. 016 C1). The posterior end (*k*) was killed when the embryo had reached the stage shown in Fig. 4; the anterior end continued to develop and has come to lie on the right side of the egg. *ab*, abdomen; *h*, head; *t*, thoracic appendages; *tf*, tail fold; *y*, *yolk*.

FIG. 18. As in Fig. 17 four days old (L.D. 016 C2).

embryo contracted and left a large yolk space (*y*) between it and the killed material (*k*). A similar condition was noted above in series L.D. 04 A3, Fig. 13 (*y*).

Fig. 18 represents an embryo (L.D. 016 C2) which was allowed to live one day longer than that just described. Here the head and first thoracic segment have continued to develop reaching a stage similar to that shown in Fig. 6. This part of the embryo has changed its orientation since the operation and now lies on the right side of the egg instead of on the ventral surface. Several other cases like this were observed in series L.D. 016 and in a number of the embryos from other series of experiments.

The last egg selected from this series was fixed three days after the operation at an age of five days. It indicates that development of the living part of the embryo proceeds up to the time of hatching.

7. Killing Parts of an Old Embryo.

The eggs used for these experiments were laid at 4 P.M. June 17, and operated upon at 4 P.M. June 20, at the age of three days. Part of them were kept as controls; the rest were divided into two lots and operated upon as indicated in Table VI: The control eggs hatched on June 22.

TABLE VI.

Experiments in Killing Parts of Old Embryos.

Leptinotarsa decemlineata—Series L.D. 06.

Number of Experiment.	Stage when Operated Upon.	Nature of Operation.	Interval between Operation and Fixation.	Remarks.
L.D. 06 A	Control			Hatched normally.
L.D. 06 B1	Old embryo (see Fig. 6).	Posterior end killed.	0	See Fig. 6.
L.D. 06 B2			1 day.	
L.D. 06 B3			2 days.	
L.D. 06 B4			4 days.	
L.D. 06 C1		Anterior end killed.	1 day.	
L.D. 06 C2			2 days.	
L.D. 06 C3			4 days.	

Fig. 6 shows a normal embryo fixed at the time of the operation. The eggs under experimentation were fixed at intervals and stained and mounted. In every case the part of the embryo that remained alive continued to develop. This was true for both those with the anterior part and those with the posterior part killed. There were no signs of regeneration even after the normal embryonic period had passed. The killed part of the embryo began to disintegrate immediately after the operation.

8. Summary.

1. If the region of a freshly laid egg of *Leptinotarsa decemlineata*, which contains the germ cell determinants (Fig. 1, *gcd*), is killed with a hot needle and these granules are thus prevented from taking part in embryonic development, the embryo produced by the rest of the egg lacks the germ cells. This supple-

ments former experiments in removing the germ cell determinants, and indicates that these granules really determine the germ cells.

2. When the primordial germ cells of *Leptinotarsa decemlineata* are killed in the blastoderm stage (Fig. 2, *pgc*) the resulting embryos lack germ cells. This is the earliest known stage in which surgical castration has been performed among the Insecta.

3. When the anterior or posterior parts of freshly laid eggs (Fig. 1) are killed, the material remaining alive develops that part of the embryo which it would have produced if the eggs had remained intact (Fig. 11). No regeneration of the part which would have been produced by the killed region takes place.

4. If the anterior or posterior parts of eggs in the blastoderm stage (Fig. 2) are killed, the resulting tissue represents the parts of the embryos which would have been produced by the living material if the entire egg had been allowed to develop (Figs. 8 and 9).

5. When parts of young embryos (Fig. 4) are killed, the remaining tissue develops normally (Figs. 15–18). Even small pieces of tissue (Fig. 17, *tf*), which are widely separated from the rest of the embryo, continue to develop normally.

6. Parts of old embryos develop up to the time of hatching. There is no regeneration of the killed part by the living tissue.

7. The eggs of *Leptinotarsa decemlineata*, at the time of deposition (Fig. 1), are definitely oriented with respect to the future position of the embryo.[1] The areas of the peripheral layer of cytoplasm (Fig. 1, *khbl*) are already set aside for the production of particular parts of the embryo, and if these areas are killed, the parts of the embryo to which they were destined to give rise will not appear. Likewise areas of the blastoderm (Fig. 2, *bl*) are destined to produce certain particular parts of the embryo.

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[1] Hegner, R. W., '09, "The Effects of Centrifugal Force upon the Eggs of Some Chrysomelid Beetles." *Journ. Exp. Zoöl.* Vol. 6.